Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 10825–10829, December 1991
Medical Sciences

Antiviral activity of 1-docosanol, an inhibitor of lipid-enveloped viruses including herpes simplex

(viral inhibition/long-chain alcohols)

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Communicated by Robert C. Gallo, August 19, 1991 (received for review July 5, 1991)

ABSTRACT **This article reports that 1-docosanol, a 22-carbon-long saturated alcohol, exerts a substantial inhibitory effect on replication of certain viruses (e.g., herpes simplex virus and respiratory syncytial virus) within primary target cells *in vitro*. To study the basis for its viral inhibitory activity, a suspension of 1-docosanol was formulated in an inert and nontoxic surfactant, Pluronic F-68; this suspension exerted potent inhibitory activity on the ability of susceptible viruses to infect cultured target cells. Susceptible viruses included wild-type herpes simplex viruses 1 and 2 as well as acyclovir-resistant herpes simplex virus 2 and also respiratory syncytial virus—all of which are lipid-enveloped. In contrast, nonenveloped poliovirus was not susceptible to the inhibitory action of 1-docosanol. Although the precise mechanism has yet to be defined, current evidence suggests that 1-docosanol inhibits viral replication by interfering with the early intracellular events surrounding viral entry into target cells. It is possible that interaction between the highly lipophilic compound and components of target cell membranes renders such target cells less susceptible to viral fusion and/or entry. If this mechanism proves to be correct, 1-docosanol may provide a broad spectrum activity against many different viruses, especially those with lipid-containing envelopes.**

Most available antiviral therapeutic compounds block replication processes shared by the virus and infected target cells (1–5). Such compounds are thus potentially toxic, mutagenic, and/or teratogenic for the host and can induce drug-resistant viral mutant substrains. Consequently, identification of efficacious new antiviral compounds that lack such deleterious effects is very important. The subject of this report, 1-docosanol, exhibits potent, broad spectrum viral inhibitory activity and its lack of toxic, mutagenic, or teratogenic properties has been well documented (6).

Previous studies have demonstrated antiviral activities of saturated and unsaturated alcohols of moderate lengths (7, 8). Optimal antiviral activity was observed with saturated alcohols 10–12 carbons long but these compounds also exhibited cytotoxic and hemolytic effects; less antiviral activity was observed with alcohols 14–18 carbons long, and alcohols of higher chain lengths were not tested (7). Studies with unsaturated alcohols and monoglycerides revealed peak activity with a triply double-bonded C-18 alcohol (8).

Because alcohols longer than 18 carbons were not examined (7, 8), we hypothesized that a molecule twice as long as C-10 or C-12 might retain antiviral activity due to folding-over of the molecule but lack the hemolytic and cytotoxic properties of the shorter molecule. Our first studies, summarized herein, have examined the saturated C-22 alcohol 1-docosanol for antiviral activity against herpes simplex virus (HSV) and other viruses in tissue culture assay systems and compare its activity on HSV to that of acyclovir (Acv; the current agent of choice for treatment of clinical HSV infections).

MATERIALS AND METHODS

Formulation of 1-Docosanol in Pluronic F-68 (Plu). 1-Docosanol (*n*-docosanol; >98% pure; M. Michel, New York) was suspended in Plu (poloxamer 188; M_r 8400; BASF, Parsippany, NJ) generally as follows. Plu was diluted to 10 mg/ml in warm (37°C) Dulbecco's high-glucose modified Eagle's medium (DMEM), and the solution was then heated to 50°C. 1-Docosanol was added at 10 mg/ml to the Plu in DMEM and the mixture was further heated to 86°C while sonicating (Branson 450 sonifier) for 21 min at an initial output of 65 W; in certain other studies, 1-docosanol and Plu were combined at higher concentrations of 20 or 100 mg/ml. The corresponding Plu control solutions were prepared identically except the 1-docosanol was excluded.

Viruses and Cell Lines. Viruses obtained from the American Type Culture Collection were as follows: (*i*) HSV-1 (MacIntyre strain, no. VR-559), (*ii*) HSV-2 (MS strain, no. VR-540), (*iii*) respiratory syncytial virus (RSV; no. VR-26), and (*iv*) poliovirus (no. VR-192). Stock preparations were titered for levels of plaque-forming units (pfu) in Vero cells (African Green monkey kidney; ATCC no. CCL-81) and stored frozen at −70°C. A laboratory isolate of Acv-resistant HSV-2 was derived by passage of wild-type HSV-2 in Vero cells in the presence of 20 μg of Acv per ml (Burroughs Wellcome). The resulting isolate (denoted LK-78) was plaque-purified by four cycles of growth in Vero cells and was completely infective for Vero cells in the presence of 20 μg of Acv per ml.

Fresh clinical samples of HSV were isolated from active lesions of patient volunteers with recurrent herpes labialis or genitalis by inoculating vesicle fluids into Vero cell monolayers. After 48 hr, virus was recovered by lightly sonicating the culture fluids (9) and then expanding in 50-ml bulk Vero cell cultures for 72 hr. The resulting supernatant fluids were lightly sonicated and the recovered viral isolates were then stored frozen at −70°C.

Virus Infection of Cell Cultures and Assays. *HSV.* Vero cells were cultured (6×10^5 cells in 1.8 ml per 35-mm well or 3×10^5 cells in 0.8 ml per 16-mm well) in DMEM supplemented with 5% fetal calf serum, sodium pyruvate, L-glutamine, penicillin/streptomycin, and 1 mM Hepes buffer at 37°C in humidified 10% CO_2. 1-Docosanol or corresponding control suspensions were added at the outset of the culture. After 24 hr, 175 (35-mm wells) or 50 (16-mm wells) pfu of HSV-1 or

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Abbreviations: Acv, acyclovir; FITC, fluorescein isothiocyanate; HSV, herpes simplex virus; pfu, plaque-forming unit(s); Plu, Pluronic F-68; RSV, respiratory syncytial virus.

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HSV-2 was added; Acv was added to such treated cultures generally at time of viral infection (see figure legends). After 42–44 hr, cultures were washed once with saline, fixed and stained with 1.25 mg of carbol-Fuchsin per ml plus 2.5 mg of methylene blue per ml in methanol, and scored for plaques. The data are averages of duplicate cultures, which varied by no more than 5–10%; statistical comparisons were made by Student's *t* test.

RSV. Vero cells (3×10^5) were cultured for 24 hr with or without inhibitor (100 infectious virions per well). After 72 hr, cultures were washed, stained, and scored for the presence of typical RSV-induced syncytia of multinucleated giant cells.

Poliovirus. Poliovirus was assayed in similar Vero cell cultures except that cultures were inoculated with only 30 pfu and were incubated for only 24 hr after addition of virus because of its very rapid growth characteristics.

Assays for HSV Antigens. *ELISA.* ELISA for core and envelope proteins (Ortho Diagnostics) was performed as originally described (10).

Fluorescence assay for immediate-early protein. The 175-kDa HSV-1 immediate-early protein ICP-4 (11) was detected in HSV-infected cells using (sequentially) (*i*) murine monoclonal anti-ICP-4 (ATCC no. HB 8183), (*ii*) biotinylated goat anti-mouse IgG, and (*iii*) fluorescein isothiocyanate (FITC)-labeled streptavidin (Zymed Laboratories). Fluorescence was scored visually with a fluorescent microscope.

RESULTS

1-Docosanol Suspended in Plu Is Nontoxic for Mammalian Cells or HSV. Biologically active, nontoxic, and stable suspensions of the water-insoluble 1-docosanol were generated by suspending 1-docosanol in the surfactant Plu, a nonionic, nontoxic aliphatic block copolymer of propylene glycol, propylene oxide, and ethylene oxide. Transmission electron microscopy of the very fine particle suspension reveals globular particles mainly 0.03 μm in diameter and ranging up to 0.3 μm. These suspensions are extremely stable; they retain their integrity when incubated with Vero cells in the HSV plaque assay, and they can be stored at 25°C for at least 8 weeks and at 37°C for 4 weeks with no appreciable loss in antiviral activity, no phase separation, and no change in particle size.

Most importantly, these suspensions do not exhibit detergent-like toxicity for either mammalian cells or viruses in culture. Thus, 1-docosanol/Plu had no appreciable effect on the normal growth or viability of cultured Vero cells (up to 100 mg/ml) or several other nucleated human and murine cell lines (up to 25 mg/ml) nor did it cause acute hemolysis of erythrocytes (100 mg/ml; 2.5 hr). Moreover, neither 1-docosanol/Plu nor control Plu suspension (5 mg/ml) exerted detectable direct virucidal effects when incubated with HSV for 2.5 hr.

1-Docosanol Inhibits *in Vitro* Replication of Wild-Type and Acv-Resistant HSV. Fig. 1 summarizes the key points of the viral inhibitory activity of 1-docosanol against HSV and its comparison to Acv. As expected, Acv inhibited plaque formation and production of infectious virions by wild-type, but not Acv-resistant, HSV-2 (Fig. 1 *Upper Left* and *Lower Left*, respectively). On the other hand, 1-docosanol exerted comparable inhibitory activity against wild-type and Acv-resistant HSV-2 (Fig. 1 *Upper Right* and *Lower Right*, respectively). This latter point has clinical significance in view of the recent increase in numbers of Acv-resistant herpes cases, particularly in immunocompromised patients who are maintained on low-dose Acv therapy for prolonged periods (12–14). In contrast, the control Plu solution was inactive at all concentrations. The ED_{50} of 1-docosanol is thus around 2.5 mg/ml for plaque inhibition and 1.7 mg/ml

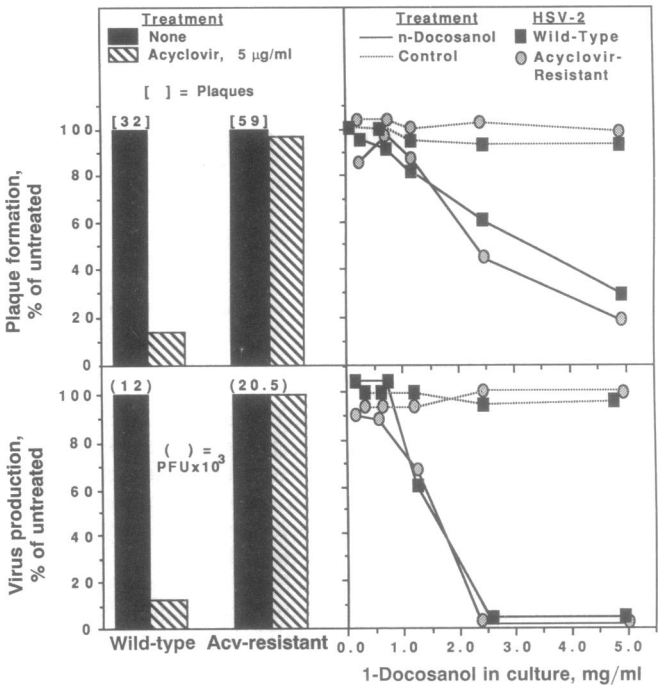

FIG. 1. 1-Docosanol inhibits plaque formation and virus production by Acv-resistant and wild-type HSV-2 isolates. Vero cells (3×10^5 per well) were cultured for 24 hr with various amounts (0.312–5.0 mg/ml) of 1-docosanol/Plu suspensions or corresponding Plu control suspensions and then infected with either 35 pfu of wild-type HSV-2 or 60 pfu of Acv-resistant HSV-2; Acv-treated cultures received 5 μg of Acv per ml at this time. After an additional 44 hr, the culture fluids were harvested to assay for infectious virus production (measured by plaque formation in secondary untreated Vero cell cultures; *Lower Left* and *Lower Right*), and the monolayers were fixed, stained, and scored for the presence of direct plaques (*Upper Left* and *Upper Right*). Data are presented as average % of treated vs. untreated controls. Statistically significant differences in *Upper Left* and *Upper Right* were as follows: None (wild-type) vs. Acv, $P = 0.0025$; vs. 1-docosanol at 2.5 and 5 mg/ml, $P \leq 0.017$; None (Acv-resistant) vs. 1-docosanol at 2.5 and 5 mg/ml, $P \leq 0.02$. Statistically significant differences in *Lower Left* and *Lower Right* were as follows: None (wild-type) vs. Acv or 1-docosanol at 2.5 and 5 mg/ml, $P \leq 0.012$; None (Acv-resistant) vs. 1-docosanol at 2.5 and 5 mg/ml, $P = 0.003$.

for inhibition of virus production for wild-type and Acv-resistant HSV-2.

Thus, the therapeutic index or selectivity ratio for 1-docosanol [LD_{50} for Vero cells (>100 mg/ml) ÷ ED_{50} for HSV inhibition (2.5 mg/ml), Fig. 1] is >40.

1-Docosanol Inhibits Replication of HSV-1. Although 1-docosanol treatment resulted in diminished production (>95%) of infectious HSV-2 virions in primary target cells (Fig. 1), it is possible that replication may have occurred but resulted in production of defective virions. However, as shown in Table 1, culture fluids from 1-docosanol-treated HSV-infected Vero cells contained 81% less of core and envelope viral proteins than were present in untreated or control cultures. Likewise, 1-docosanol substantially reduced the numbers of infected cells expressing the intranuclear 175-kDa HSV-1-specific immediate-early protein (ICP-4) within 6 hr after infection. Thus, 1-docosanol significantly inhibits early stages of viral replication and the consequential production of viral proteins, including those (ICP-4) synthesized at very early nuclear phases of replication.

Inhibition of viral replication by 1-docosanol is also corroborated by the diminished size of viral plaques (mean diameter per 10 plaques) observed in 1-docosanol-treated (0.47 ± 0.18 mm) or Acv-treated (0.36 ± 0.12 mm) HSV-2 cultures versus control-treated cultures (1.07 ± 0.26 mm).

Table 1. 1-Docosanol inhibits HSV-1 protein synthesis in primary target cells

Inhibitor added to culture	HSV-1 antigens,* units/ml × 10^3	Nuclear protein,† % ICP-4$^+$ cells
None	100	27.2 ± 6
Control Plu suspension‡	120	27.1 ± 2.6
1-Docosanol suspension‡	19§	8.7 ± 2.8¶

*Vero cells (3×10^5 per ml) were cultured for 24 hr in medium alone, 1-docosanol/Plu, or control Plu suspension and then infected with 5×10^5 pfu of HSV-1 per well. After a further 44 hr, the supernatant fluids were harvested and tested for the quantities of infectious virus by plaque formation in secondary Vero cell cultures (see Fig. 1 legend) and for HSV antigens by ELISA. Quantitation of infectious virions revealed >99% inhibition of infectious viral units in cultures treated with 1-docosanol (not shown). The amount of HSV-1 core and envelope protein antigens detectable in duplicate primary culture fluids was quantitated by ELISA (10).

†Vero cells (6×10^5 per ml) were cultured on glass coverslips in 35-mm dishes for 24 hr in medium alone, 1-docosanol/Plu, or control Plu suspension and then infected with HSV-1 at a multiplicity of infection of 0.33 infectious particle per cell. After 6 hr, the cultures were washed, fixed, and stained for the presence of the 175-kDa HSV-1 immediate-early protein ICP-4. Shown are the average (±SD) % of cells (from multiple counts of duplicate samples) positive for nuclear fluorescence.

‡5 mg/ml.

§81% inhibition.

¶68% inhibition.

Plaque formation is dependent on adjacent cell-to-cell infection by virus released from the initial target cell. Plaque formation by HSV-2-infected Vero cells planted onto fresh Vero cell monolayers (infectious center assay) was also inhibited by pretreatment with 1-docosanol (not shown). The fact that 1-docosanol inhibits plaque formation and plaque size explains the apparent discrepancy of lesser total plaque inhibition relative to the marked reduction in virus production.

1-Docosanol Inhibits Plaque Formation by Fresh Clinical Isolates of HSV. To rule out the possibility that the laboratory strains of HSV used herein might be uniquely sensitive to 1-docosanol, its activity on replication of HSV freshly isolated from patients with active lesions was tested. As shown in Fig. 2, a clinical HSV oral isolate (LK-117) was inhibited by 1-docosanol (>90%) and Acv (100%). Comparable results were obtained with fresh isolates from herpes genitalis lesions (not shown).

Studies on Mechanism(s) of Action of 1-Docosanol. *Optimal inhibitory activity requires preincubation with target cells.* As stated above, direct preincubation of HSV with 1-docosanol does not affect infectivity of recovered virus. In contrast, optimal inhibitory activity requires that Vero cells be preincubated with 1-docosanol for sufficient time before virus is added. Thus, addition of 1-docosanol to Vero cells either concurrently with HSV or from 0.5 to 6 hr before HSV generally results in only around 30–40% inhibition and rarely exceeds 50%. In contrast, when preincubation with 1-docosanol exceeds 12–15 hr (24 hr is standardly employed), substantially greater inhibition (75–95%) is obtained. This time-dependent preincubation requirement is probably related to the slow uptake of the compound by target cells (see below).

Binding and uptake of 1-docosanol by Vero cells is slow and progressive. Because 1-docosanol is lipophilic, it should associate with cell membranes. Exchange of lipoidal components from vesicles to cells in culture is characteristically slow, which would explain the time dependence of target cell preincubation essential for optimal viral inhibitory activity. 1-[^{14}C]-Docosanol was used to analyze the kinetics and stoichiometry of uptake by cultured Vero cells and confirmed the following. (*i*) There is a concentration-related effect

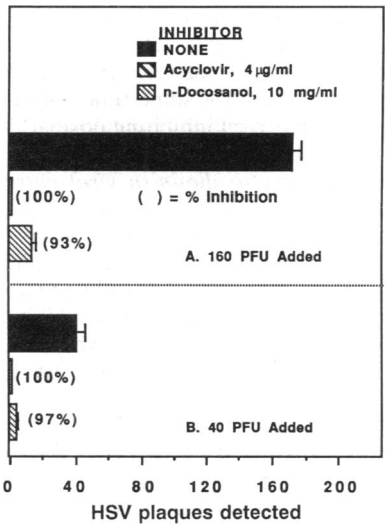

FIG. 2. 1-Docosanol inhibits plaque formation by freshly isolated HSV. Vero cells (6×10^5 per well) were cultured for 24 hr with either Acv (4 μg/ml) or 1-docosanol (10 mg/ml)/Plu (or left untreated) and then inoculated with 160 (*A*) or 40 (*B*) pfu of freshly isolated HSV (LK-117). Differences were statistically significant as follows: (*A*) 160 pfu, None vs. Acv, $P = 0.001$; None vs. 1-docosanol, $P = 0.002$. (*B*) 40 pfu, None vs. Acv, $P = 0.034$; None vs. 1-docosanol, $P = 0.03$.

during the 24-hr preincubation time period, which peaks at 1 mg/ml (Table 2). (*ii*) 1-Docosanol uptake is slow and progressive, remaining relatively flat from 30 min to 4 hr and then doubling by 24 hr after initial exposure (Table 2). Because cells are proliferating in these cultures, the per cell binding was determined at 4-hr intervals and revealed that maximal binding of 1-docosanol occurs between 8 and 12 hr (not

Table 2. Kinetics and concentration dependence of 1-[^{14}C]docosanol binding to cultured Vero cells

Concentration dependence (24 hr)*		Kinetics†	
Amount added, mg/ml	1-[^{14}C]Docosanol bound, μg per well	Incubation time, hr	1-[^{14}C]Docosanol bound, μg per well
0.125	2.2	0.25	0.58
0.25	3.3	0.50	1.20
0.50	4.8	1	1.30
1.00	5.1	2	1.44
2.00	4.1	4	2.10
3.00	4.6	6	2.97
		24	4.20

Values shown are the average of duplicates and have been corrected for nonspecific binding to the plate by subtracting values obtained in control wells lacking cells.

*1-[^{14}C]Docosanol (specific activity, 55 mCi/mmol; American Radiolabeled Chemicals, St. Louis) was included as a tracer mixed with unlabeled material in preparing 1-docosanol/Plu suspensions. Vero cells (6×10^5 per well) were cultured for 24 hr with various quantities of 1-[^{14}C]docosanol. The suspension was then removed, the plates were washed extensively with saline, and the cells were solubilized for scintillation spectroscopy by trypsinization with 10 μg of trypsin per ml for 15 min at 37°C. Cell-associated 1-[^{14}C]docosanol was quantitated by scintillation spectroscopy. The amount of 1-docosanol associated with the cells is not diminished by extensive or prolonged (40 min, 37°C) washing with 0.25 M cesium bromide, 0.9% saline, or DMEM, indicating that the molecule is tightly bound to Vero cells.

†1-[^{14}C]Docosanol/Plu suspension (1 mg/ml) was incubated with Vero cells for the times indicated, the suspension was removed, and the amount of cell-associated 1-[^{14}C]docosanol was determined by scintillation spectroscopy.

shown). (*iii*) Even at optimal conditions, <1% of the total 1-docosanol added actually interacts tightly with the attached Vero cell monolayer. This indicates that high quantities of 1-docosanol must interact for some time with cultured cells to deliver the effective viral inhibiting dose at the target cell level.

1-Docosanol appears to diminish viral entry into target cells. The 1-docosanol-related preconditioning appears to prevent or markedly diminish entry of HSV-2 into target cells. The evidence for this is 2-fold: (*i*) in addition to target cell preconditioning, 1-docosanol must be present at the time of initial exposure of cells to HSV since inhibition is considerably lessened if the compound is removed from the cultures immediately prior to HSV infection (Fig. 3*A*) and (*ii*) 1-docosanol exerts most of its inhibitory effect during the early hours after HSV-2 infection, since removal of the compound 16 hr after adding virus to the target cells does not diminish its viral inhibitory activity (Fig. 3*B*).

1-Docosanol does not block HSV receptors. The possibility that 1-docosanol blocks binding of HSV to its target cell receptors was directly tested using [^{3}H]thymidine-labeled HSV [internally labeled by infecting Vero cells at a multiplicity of infection of 0.5 pfu per cell in the presence of 2 μCi of [^{3}H]thymidine per ml (1 Ci = 37 GBq) for 2 days and recovering radiolabeled virus by differential centrifugation]. Uptake of ^{3}H-labeled HSV-1 (5×10^5 pfu per 2.5×10^5 cells per 16-mm culture) after 1 or 2.5 hr of exposure to Vero cells that had been preincubated overnight with medium alone, 1-docosanol, or control Plu suspension was determined after unbound radioactivity was washed away, the adherent cells were isolated by trypsinization (10 μg of trypsin per ml for 15 min at 37°C), and the cell-bound ^{3}H was assayed by liquid scintillation. This analysis revealed that binding of ^{3}H-labeled HSV to Vero cells (1 hr = ≈3000 cpm; 2.5 hr = ≈22,000 cpm) is unaffected by treatment with 1-docosanol (or Plu control)—i.e., the compound does not block HSV-specific receptor sites. The positive control for this study confirmed that uptake/binding of ^{3}H-labeled HSV-1 to Vero cells was specifically inhibited (>95%) by murine anti-HSV-1 antibodies (but not by normal mouse serum).

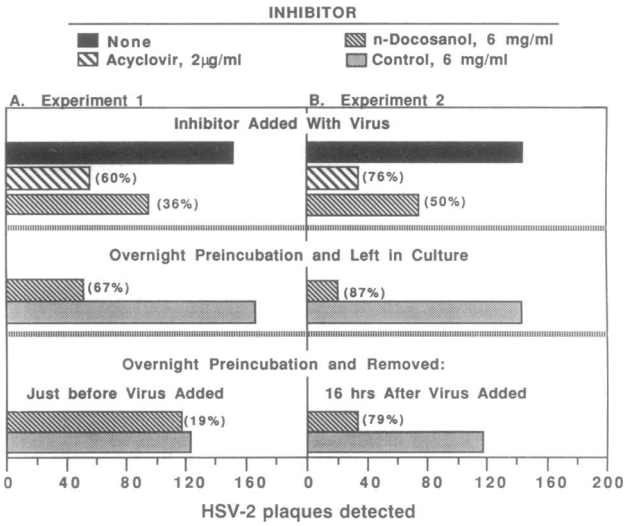

FIG. 3. Optimal 1-docosanol inhibitory activity requires preincubation of target cells and the presence of 1-docosanol at the time of infection. Two separate experiments are shown. In both experiments, the top three bars show cultures in which the inhibitors (Acv or 1-docosanol) were added together with inoculation of 6×10^5 Vero cells with 175 pfu of HSV-2. In the middle sections of each panel, 1-docosanol/Plu or the control Plu suspension was preincubated with target cells for 24 hr prior to addition of virus and left in culture throughout. In the bottom portion of *A*, the treated cells were preincubated for 24 hr and then the 1-docosanol or Plu was replaced with fresh medium immediately prior to inoculation with HSV-2. In the bottom portion of *B*, the 1-docosanol/Plu or control Plu suspension was preincubated with cells for 24 hr and left in for 16 hr after HSV-2 addition and then removed and replaced with fresh medium for the duration of the cultures. Parentheses depict the % inhibition observed in treated vs. untreated cultures. Statistically significant differences were as follows: (*A*) None vs. Acv, $P \leq 0.01$; None vs. 1-docosanol (67% value), $P \leq 0.01$. (*B*) None vs. Acv or any 1-docosanol value, $P \leq 0.009$.

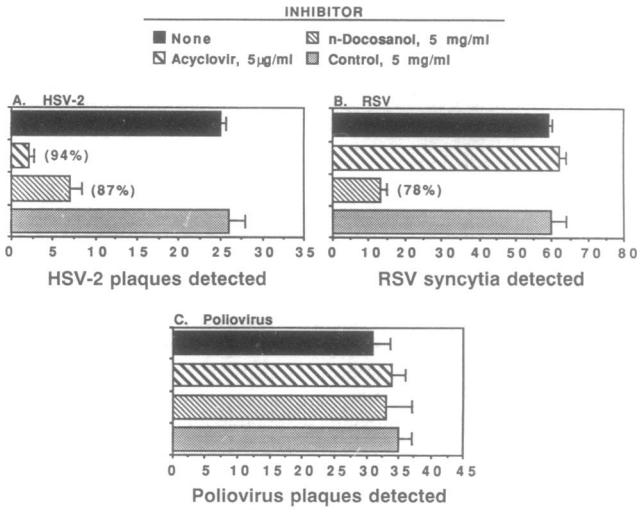

FIG. 4. 1-Docosanol exhibits preferential inhibitory activity for lipid-enveloped viruses. Vero cells (3×10^5 per well) were cultured for 24 hr in medium alone or with Acv (5 μg/ml), 1-docosanol (5 mg/ml)/Plu, or control Plu solution (5 mg/ml) and then infected with either 50 pfu of HSV-2 (*A*), 100 syncytia-forming units of RSV (*B*), or 30 pfu of poliovirus (*C*). The harvested cultures were stained and scored for plaques (*A* and *C*) and syncytia (*B*). Parentheses indicate the % inhibition in treated vs. untreated cultures. Differences were statistically significant as follows: (*A*) None vs. Acv, $P = 0.002$ and None vs. 1-docosanol, $P = 0.006$. (*B*) None vs. 1-docosanol, $P = 0.0012$.

1-Docosanol exhibits preferential inhibitory activity for lipid-enveloped viruses. Since it is highly lipophilic, 1-docosanol may exert inhibitory activity for several viruses that are lipid-enveloped and utilize this property as a "doorway" into target cells via membrane fusion. If so, then nonenveloped viruses might be less or nonsusceptible to its inhibitory activity. The experiment presented in Fig. 4 compares inhibitory activity of 1-docosanol for HSV to that against RSV (an enveloped virus) and nonenveloped poliovirus. Three points are noteworthy about these data. (*i*) Acv inhibits HSV replication (Fig. 4*A*) but has no effect on either RSV (Fig. 4*B*) or poliovirus (Fig. 4*C*); this result conforms to the expected selectivity of action of this drug (for herpesvirus DNA polymerase). (*ii*) Similar to its inhibitory effects on HSV-2 (Fig. 4*A*), 1-docosanol clearly exerts inhibitory activity against primary syncytia formation and viral replication by RSV (Fig. 4*B*). (*iii*) Conversely, 1-docosanol fails to inhibit primary plaque formation or viral replication by poliovirus (Fig. 4*C*), thus supporting the possibility that 1-docosanol might lack inhibitory activity for a nonenveloped virus.

DISCUSSION

The antiviral activity of a long-chain, saturated fatty acid was initially reported 50 years ago (15). These studies were extended more recently when Snipes and colleagues (7, 8) reported that preincubation of certain viruses with saturated or unsaturated fatty alcohols of moderate lengths prior to infecting target cells resulted in antiviral effects. Their interpretation was that these effects resulted from attachment of

hydrophobic regions of the alcohols to lipid components of the virus membranes while simultaneously orienting themselves at the membrane–aqueous interface through the molecules' polar groups (7, 8). Subsequently, Clark and colleagues (16) concluded that the 30-carbon-long saturated alcohol triacontanol was active against herpes simplex infections, but it was speculated that the antiherpes activity observed in animal studies reflected an immunostimulatory effect of this compound (17).

The present studies document that the 22-carbon-long saturated alcohol 1-docosanol substantially inhibits replication of HSV and RSV within primary target cells. This water-insoluble compound was formulated in an inert, nontoxic vehicle, Plu, that preserved bioavailability of the active moiety, while the vehicle alone displayed no evidence of viral-inhibitory (or toxic) effects. The possibility that the 1-docosanol might render the otherwise inert vehicle components active—i.e., virucidal—appears to be excluded by the fact that such suspensions exerted no detergent-like activity in (*i*) mammalian cell cultures, (*ii*) erythrocyte suspensions, or (*iii*) direct contact with infectious HSV.

The mechanism by which 1-docosanol inhibits viral replication is clearly different from that of Acv or other drugs (such as phosphonoformate, phosphonoacetic acid, and adenine arabinoside) that inhibit herpesvirus DNA polymerase activity (18) since it inhibits Acv-resistant mutants of HSV-2 (Fig. 1) and Acv-insensitive RSV (Fig. 4). Although its mechanism of action has yet to be precisely defined, certain observations narrow down the possibilities. First, 1-docosanol does not directly disrupt or inactivate virus since (*i*) preincubation of virus with the compound does not diminish infectivity and (*ii*) the compound does not prevent binding of radiolabeled virus to target cells, indicating that virus is still intact and that HSV receptors are neither specifically nor sterically blocked.

Second, optimal inhibitory activity requires preconditioning of target cells with 1-docosanol and its presence during early stages of virus–target cell interactions *in vitro*, although the compound can be removed once the peak period of viral entry is complete. Third, these interactions between the highly lipophilic compound and components of either or both of the target cell and/or viral particle membranes markedly diminish viral replication, suggesting that the compound somehow interferes with the early intracellular events surrounding viral entry into target cells.

Ongoing studies suggest that how 1-docosanol associates with target cells and then exerts viral inhibitory activity probably involves complicated and yet-to-be determined events of membrane metabolism and fluidity. Determination of its possible effects on cellular phagocytosis and receptor-mediated endocytosis, among other things, awaits further investigation. Regarding general binding of 1-docosanol to cells, measurement of release rates indicates that the compound becomes relatively tightly associated once binding has occurred (Table 2). The slow kinetics of target cell association observed is probably a thermodynamic artifact of the combined effects of the Plu formulation and the tissue culture conditions employed; this is probably why such excess quantities of compound are required to drive the small quantity of bioavailable material to observed activity *in vitro*. In fact, the bioactive dose is actually in the 2.5 μg/ml range (Table 2).

1-Docosanol may interact with, and possibly stabilize, lipids in the target cell membranes, thereby rendering them less susceptible to viral fusion and entry [note, a C-20 aliphatic chain has approximately the correct length (*ca.* 30 Å) to span one-half of the cell membrane bilayer]. There may also be critical interactions with lipids in the viral envelope, but apparently only *after* viral attachment (which is not affected by 1-docosanol) to the target cell has occurred (this would explain why the 1-docosanol must be present concomitantly during the earliest stages of viral infection). Since initial uncoating of viral particles depends on early enzymatic digestion within the target cell membrane (9), the net result of these 1-docosanol/membrane lipid interactions could be either diminished or premature uncoating of such viral particles. However, we cannot exclude additional effects of 1-docosanol on postreplicative events.

If this hypothesis proves true, then 1-docosanol could become a significant new viral inhibitory drug with a potentially broad spectrum for several different viruses, especially those with lipid-containing envelopes, and low host toxicity. Moreover, the fact that 1-docosanol inhibits adjacent cell-to-cell infection, as evidenced by decreased plaque size and inhibition of infectious centers, suggests that it would be effective in already established infections.

Its potentially broad activity against lipid-containing viruses is supported by the demonstrated inhibitory effects on two enveloped DNA viruses of the herpesvirus family, HSV-1 and -2, and the enveloped RNA virus, RSV; conversely, 1-docosanol lacked activity against another RNA, but nonenveloped, virus, poliovirus. Other ongoing studies indicate that 1-docosanol may inhibit two additional enveloped murine retroviruses; future work is necessary to examine its potential inhibitory activity against pertinent human retroviruses.

Finally, the demonstration that 1-docosanol exerts strong inhibitory activity against Acv-resistant HSV *in vitro* indicates its potential as a candidate drug for human patients so infected.

We thank Regina McFadden and Jing Wang for excellent technical assistance, Beverly Burgess, Tricia Brehaut, and Danette Stroh for skillful assistance with the manuscript, and Drs. R. T. Ogata, M. F. Mescher, C. O. Cowing, A. M. Kleinfeld, D. E. Mosier, M. L. Richards, S. Zebedee, S. Straus, and F. M. Huennekens for critically reviewing and commenting on the studies and/or the manuscript. This is publication no. 206 from Medical Biology Institute and no. 2 from LIDAK Pharmaceuticals.

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